REMARKS

Claims 1-6, 9-14 and 17-19 were pending in the present application. For the Examiner's convenience, a copy of the claims as currently pending herein is attached hereto as Appendix A. No new matter has been added.

Double Patenting Rejection of Claims 1-6, 9-14, and 17-19

Claims 1-6, 9-14, and 17-19 are rejected under the judicially created doctrine of nonstatutory obvious-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 5,888,981. Applicants will submit a Terminal Disclaimer in compliance with 37 C.F.R. 1.321(c) when the claims are otherwise indicated allowable.

Rejection of Claims 1-6, 9-14, and 17-19 under 35 U.S.C. § 112, First Paragraph

Claims 1-6, 9-14, and 17-19 are rejected under 35 U.S.C. § 112, first paragraph, because according to the Examiner, "the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims." Specifically, the Examiner states that the claimed invention is not enabled, "because the method of targeted expression of a nucleic acid is highly unpredictable."

Applicants respectfully traverse this rejection. Applicants point out that the specification provides ample guidance to enable one of ordinary skill in the art to make and use the invention.

The claimed invention pertains to a method for regulating expression of a *tet* operator-linked gene in a cell of a subject using the tetracycline-controllable transactivator, referred to herein as the tTA system. The method of the invention consists of introducing into the cell a first nucleic acid comprising the *tet* operator-linked gene, and introducing into the cell a second nucleic acid molecule encoding a tetracycline-controllable transactivator (tTA). The tTA comprises a Tet repressor operably linked to a

polypeptide which directly or indirectly activates transcription in eucaryotic cells. The first and second nucleic acids of the invention are not covalently linked to each other. Furthermore, the concentration of tetracycline, or analogue thereof, in the subject can be modulated.

The specification provides significant guidance regarding each component of the tTA system, describing specific preferred examples (see *e.g.* pages 12-14 and 25-27). Additionally, suggestions of alternative components which may be more applicable depending for example, on a particular cell line, are provided. At page 30, lines 29-33, for example, the specification states that, "the tissue specificity of some promoters dictate that the tet operator sequence/promoter sequence fusion has to be designed with the particular application and cell line in mind following the teachings in this application using promoters customarily used for the cell line in question." These alternative components may readily be substituted using standard molecular biology techniques known to those skilled in the art.

The specification provides detailed description on how to make nucleic acid molecules encoding the transcriptional activator fusion proteins of the invention, including specific examples (see *e.g.*, pages 12-13, 25-26 and Example 1). Furthermore, the specification states the use of the tTA system in several cell lines and provides examples of suitable cell lines and citations for expression of recombinant proteins in the cell lines (see page 15-16).

Transcription activation fusion proteins made using the methods described in Example 1 were transfected into Hela cells to demonstrate *ex-vivo* modification of the cell response in cultured cells grown in the presence or absence of Tc, based on regulation of luciferase expression. This demonstrates a working example of Tc regulated expression of luciferase which is reproducible and that causes a five fold increase in the expression of luciferase (see *e.g.*, pages 45-48). One of ordinary skill in the art would recognize that these results demonstrate the application of the tTA system in regulating individual genes in higher eukaryotic cells.

Moreover, Applicants point out that the tTA system of the invention has successfully been used in many scientific publications to modify a variety of host cell

types *ex vivo*. A sample of 12 of these publications are listed below. Copies of these references were provided with the Information Disclosure Statement filed on October 6, 1999.

1) Agarwal *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:8493-8497, which uses the tTA system in human fibroblasts (see ref. CU in IDS);

2) Bergman *et al.* (1995) *Mol. Cell Biol.* 15:711-722, which uses the tTA system is HeLa cells (see ref. BS in IDS);

3) Buckbinder *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:10640-10644, which uses the tTA system in human osteosarcoma cells (see ref. BT in IDS);

4) Cayrol and Flemington (1995) *J. Virol.* 69:4206-4212, which uses the tTA system in epithelial cells (see ref. DB in IDS);

5) Chen *et al.* (1995) *Cancer Research* 55:4536-4539, which uses the tTA system in colon carcinoma and prostate carcinoma cells (see ref. DC in IDS);

6) Gjetting *et al.* (1995) *Biol. Chem. Hoppe-Seyler* 376:441-446, which uses the tTA system in mammary carcinoma cells (see ref. DP in IDS);

7) Haase *et al.* (1994) *Mol. Cell. Biol.* 14:2516-2524, which uses the tTA system in human embryonic kidney fibroblasts (see ref. CC in IDS);

8) Howe *et al.* (1995) *J. Biol. Chem.* 270:14168-14174, which uses the tTA system in rat pituitary-derived cells (see ref. EA in IDS);

9) Miller and Rizzino (1995) *Exp. Cell Res.* 218:144-150, which uses the tTA system in embryonal carcinoma cells (see ref. CF in IDS);

10) Resnitzky *et al.* (1994) *Mol. Cell. Biol.* 14:1669-1679, which uses the tTA system in Rat-1 fibroblasts (see ref. CH in IDS);

11) Sopher *et al.* (1994) *Mol. Brain Res.* 26:207-217, which uses the tTA system in human neuroblastoma cells (see ref. CJ in IDS); and

12) Wu *et al.* (1995) *Genes Dev.* 9:2350-2363, which uses the tTA system in NIH-3T3 cells (see ref. CL in IDS).

The above-references are representative examples of numerous references available in the art that show the ability of the tTA system to function *ex vivo* in many different host cell types.

In addition to the *ex vivo* use of the tTA system, the specification also provides ample guidance for its use in *in vivo* systems. For example, use of the tTA system to create "conditional knockouts" whereby expression of a gene of interest is switched off by the tTA system (see page 18 to 19). This can be accomplished, for example, by using the tTA system and a process of homologous recombination in ES cells. ES technology was known in the art at the time of the invention with several published articles available in the art that describe using this technique. Furthermore, homologous recombination was also known and successfully used to generate several species with genes either at random or specific locations within a chromosome, at the time of the invention.

The specification describes how to prepare transgenic organisms by homologous recombination such that the transgene is integrated at a predetermined location in the genome. Guidance is provided regarding the vectors required for homologous recombination (see *e.g.*, pages 19-24 and 28-29). Additionally, the specification cites, and incorporates, several references describing homologous recombination methodologies a leading article on the subject (see *e.g.*, page 19, lines 35-38), stating that these methodologies are well established in the art.

The specification also describes using the tTA system to generate a transgenic animal which contains in its genome a gene of interest under the transcriptional control of the Tc responsive promoter element. This can be accomplished as described at page 20 The specification further provides guidance regarding how to incorporate such nucleic acid molecules into vectors for making transgenic or homologous recombinant organisms (see *e.g.*, pages 19-24 and 28-29) and how to prepare such transgenic and homologous recombinant organisms (see *e.g.*, pages 19-24 and 28-29 and Example 2). The

specification still further describes how to operatively link a gene of interest to a *tet* operator sequence(s) (see *e.g.*, pages 14, 16 and 27). Moreover, the specification describes how to regulate transcription of a gene of interest using the transcriptional activator fusion protein (see *e.g.*, pages 29-31).

As discussed in the specification, standard molecular biology techniques are used to construct the DNA and vectors of the invention. Furthermore, as discussed in the specification, standard techniques known in the art are used to prepare the transgenic organisms of the invention.

Moreover, the specification provides examples of successfully using the tTA system in a transgenic organism, e.g. mice (see Example 2). This demonstrates that the tTA effectively stimulates the expression of a reporter gene operably linked to a tTA responsive promoter in multiple tissues of the animal *in vivo* in the absence of Tc, whereas the expression of the gene is inhibited when Tc is administered to the animals.

Moreover, the successful use of the tTA system *in vivo* has also been demonstrated in a variety of tissue types (e.g., skeletal muscle, cardiac muscle, skin, secretory tissues, pancreatic β cells) in many published articles, representative examples of which were provided in the previously submitted IDS and are summarized below.

1) Dhawan *et al.* (1995) *Somatic Cell and Molecular Genetics* 21:233-240 (see ref. BW in IDS). This study shows that expression of the tTA of the invention and a tet-responsive plasmid injected directly into mouse skeletal muscle are regulated by Tc administered orally or parenterally. The reporter gene was suppressed by two orders of magnitude in the presence of Tc and that suppression was reversed when Tc was withdrawn. These results demonstrate effective regulation of gene expression *in vivo* in skeletal muscle using the tTA system of the invention.

2) Efrat *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:35767-3580 (see ref. BX in IDS), which describes successful use of the tTA system in mouse pancreatic β cells *in*

vivo, and suggests that the system provides an approach for the generation of β-cell lines for cell therapy of diabetes.

3) Fishman *et al.* (1994) *J. Clin. Invest.* 93:1864-1868 (see ref. BZ in IDS), which describes us of the tTA system with a tet operator-linked luciferase reporter gene *in vivo,* through direct DNA injection into hearts of adult rats. Cardiac luciferase activity increased over two orders of magnitude in response to small changes in input tetracycline-controlled transactivator DNA. The study demonstrate effective regulation of gene expression *in vivo* in cardiac muscle using the tTA system of the invention and further show that the extent of gene expression *in vivo* can be rapidly and reversibly controlled by manipulating tetracycline treatment *in vivo*.

4) Henninghausen *et al.* (1995) *J. Cell. Biochem.* 59:463-472 (see ref. CD in IDS), which uses the tTA system in secretory tissue and skin of mice *in vivo*. This study demonstrates several hundredfold activation of gene expression, which was abrogated in the presence of therapeutic levels of tetracycline.

5) Passman and Fishman (1994) *J. Clin. Invest.* 94:2421-2425 (see ref. CG in IDS), which uses the tTA system in cardiac muscle cells *in vivo*, and demonstrates the feasibility of tightly controlling the timing and extent of expression of transgenes *in vivo*.

The results presented in Example 2, in conjunction with the above citations, show not only the successful regulation of gene expression by the tTA system in multiple cell types *in vivo,* but also that the gene of interest is expressed at measurable levels and that the level of gene expression can be manipulated by adjusting the amount of tetracycline present. This sets forth the precedence and credibility for regulation of genes *in vivo* in a similar manner for therapeutic purposes.

The Examiner cites Miller *et al.* in support of the argument that, "in the area of *in vivo* gene transfer, vector targeting *in vivo* to desired organs continues to be unpredictable and inefficient." Miller *et al.* describes and evaluates various viral gene delivery systems,

including retroviral, adenoviral, liposome, and molecular conjugate vectors, and "review[s] the progress in gene delivery systems to specific cell populations," (page 190, first column). The Examiner also cites Deonarain in further support of the difficulties encountered with gene delivery strategies in gene therapy. Deonarain describes drawbacks of various targeting vectors. Regarding Applicants' enablement for the use of said invention in gene therapy, the Examiner states that "…an artisan of skill would have to carry out extensive experimentation to figure out conditions that would have allowed targeted deliveries of both the nucleic acids to the same cells."

In response, Applicants submit that the future of gene therapy is often debated and there are numerous publications both in support of gene therapy as a viable treatment as well those which cast doubt on the feasibility of gene therapy, such as that cited by the Examiner. Applicants respectfully submit that the scope of the invention should not be limited by the potential difficulties involved in gene therapy and gene delivery systems, nor by the Examiner's anticipated failure of the procedure. One skilled in the art would know how to incorporate the nucleic acids of the invention into a gene delivery system. As the claimed invention can be used for gene therapy as a regulatory system for modulating levels of gene expression (see pgae 32, lines 18-20), targeting of the nucleic acids would be dependent upon the use of the invention and is not encompassed by the scope of the claims. For example, if a skilled artisan chose to express a certain gene in a population of cells deficient for that gene, the skilled artisan would choose a delivery system that would target the nucleic acids of the claimed invention to that specific cell population. In other words, much would depend on the gene that is to be expressed under the control of the tTA system and one skilled in the art would recognize the appropriate methods to direct the nucleic acids to the cell of interest. The claimed invention does not require 100% efficiency, but anticipates that a certain percentage of cells will receive both nucleic acids.

The specification fully enables one of ordinary skill in the art to use said invention in gene therapy, describing in detail how to use the tTA system for regulation of a gene of interest in gene therapy for several disease states (see pages 32-36). Additionally, the specification (at page 32, lines 11-13) cites, and incorporates by reference, several publications describing the state of the art in gene therapy. These references describe, for example, preferred vectors and delivery systems for particular target cell types. Thus, these publications provide extensive guidance to one of ordinary skill in the art regarding the necessary physical and chemical methods that can be used for gene therapy.

In order to meet the enablement requirement, it is not necessary that a patent specification include specific examples of every different embodiment encompassed by the claims. Moreover, the fact that some experimentation may be necessary to produce a gene delivery system to be used to deliver the nucleic acids of the invention, does not constitute lack of enablement as long as the amount of experimentation is not unduly extensive. *Amgen Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1213 (CAFC 1991). A considerable amount of experimentation is permissible if it is merely routine, or if the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands* 8 USPQ2d 1400-1407, 1404 (CAFC, 1988).

In view of the teachings in the specification and the general knowledge in the art, the specification has provided sufficient guidance to the ordinarily skilled artisan as to how to make and use the invention. Accordingly, the specification meets the enablement requirement and respectfully request that the rejection of claims 1-6, 9-14, and 17-19 under U.S.C. 112 first paragraph be withdrawn.

SUMMARY

In view of the foregoing remarks, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

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